Protocol for Gel Purification

with AxyPrep DNA Gel Extraction Kit

1. Excise the agarose gel slice containing the DNA fragment of interest with a clean, sharp scalpel under ultraviolet illumination. Briefly place the excised gel slice on absorbent toweling to remove residual buffer. Transfer the gel slice to a piece or plastic wrap or a weighing boat. Mince the gel into small pieces and weigh. In this application, the weight of gel is regarded as equivalent to the volume. For example, 100 mg of gel is equivalent to a 100 µl volume. Transfer the gel slice into a 1.5 ml microfuge tube.

Note: Alternatively, the gel slice can be placed into the 1.5 ml microfuge tube and then crushed with a pipette tip or other suitable device. Spin the tube for 30 sec at 12,000xg to consolidate the gel at the bottom of the tube. Use the graduations to estimate the volume of the agarose gel.

2. Add a 3x sample volume of Buffer DE-A.

Note: The color of Buffer DE-A is red. This color is used to add contrast in the next step, so that any pieces of unsolubilized agarose can be visualized.

3. Resuspend the gel in Buffer DE-A by vortexing. Heat at 75°C until the gel is completely dissolved (typically, 6-8 minutes). Heat at 40°C if low-melt agarose gel is used. Intermittent vortexing (every 2-3 minutes) will accelerate gel solubilization.

IMPORTANT: Gel must be completely dissolved or the DNA fragment recovery will be reduced.

IMPORTANT: Do not heat the gel for longer than 10 minutes.

4. Add 0.5x Buffer DE-A volume of Buffer DE-B, mix. If the DNA fragment is less than 400 bp, supplement further with a 1x sample volume of isopropanol.

Example: For a 1% gel slice equivalent to 100 µl, add the following:

- 300 µl Buffer DE-A
- 150 µl Buffer DE-B

If the DNA fragment is <400 bp, you would also add:

- 100 µl of isopropanol.

Note: The color of the mixture will turn yellow after the addition of Buffer DE-B. Please make sure the contents are a uniform yellow color before proceeding.

5. Place a Miniprep column into a 2 ml microfuge tube (provided). Transfer the solubilized agarose from Step 4 into the column. Centrifuge at 12,000xg for 1 minute.

6. Discard the filtrate from the 2 ml microfuge tube. Return the Miniprep column to the 2 ml microfuge tube and add 500 µl of Buffer W1. Centrifuge at 12,000xg for 30 seconds.

7. Discard the filtrate from the 2 ml microfuge tube. Return the Miniprep column to the 2 ml microfuge tube and add 700 µl of Buffer W2. Centrifuge at 12,000xg for 30 seconds.

Note: Make sure that 95-100% ethanol has been added into Buffer W2 concentrate. Make a notation on the bottle label for future reference.

8. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Add a second 700 µl aliquot of Buffer W2 and centrifuge at 12,000xg for 1 minute.

Note: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions, such as ligation and sequencing reaction.

9. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Centrifuge at 12,000xg for 1 minute.

10. Transfer the Miniprep column into a clean 1.5 ml microfuge tube (provided). To elute the DNA, add 25-30 µl of Eluent or deionized water to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000xg for 1 minute.

Note: Pre-warming the Eluent at 65°C will generally improve elution efficiency.

Note: Deionized water can also be used to elute the DNA fragments.

Reference: Axygen® AxyPrep™ DNA Gel Extraction Handbook